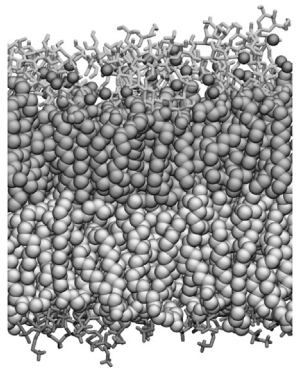
untransfected cells retained high levels of this drug indicating that the MRP1 protein is functional. Using fluorescence recovery after photobleach (FRAP) we also demonstrated that the 2-color MRP1 is freely diffusible within the plasma membrane. 2-color MRP1 exhibited dynamic FRET changes in response to ATP and ATP + substrate but not by substrate alone. FRET changes were quantified as an index of MRP1 conformational changes. These FRET changes correlate well with the available crystal structures which show close interaction of NBDs in the presence of ATP. Furthermore, we showed that ATP increased FRET in a concentration dependent manner with an apparent affinity of 107 μM. The data suggested that the relative affinity of MRP1 for nucleotides was ATP > ADP >> AMP. Finally, interactions of ATP analogs (ATPγS, AMP-PNP, AMP-PCP) with MRP1 revealed their lower affinity compared to ATP, since much higher amounts were required to induce the NBD closure. Our results provide insight into the structural dynamics of the MRP1 tranporter.

1292-Pos Board B22

Probing the Outer Membrane of Pseudomonas Aeruginosa using Molecular Dynamics Simulations

Jamie Parkin[1], Tim Carpenter[2], Syma Khalid[1].
[1]Chemistry, University of Southampton, Southampton, United Kingdom, [2]Lawrence Livermore National Laboratory, Livermore, CA, USA.

Pseudomonas aeruginosa (PA) is a pathogenic bacterium that can be fatal in persons with compromised immune systems. Increasing resistance to current antibiotics has generated a requirement to develop not only novel drug design methods, but a greater understanding of the drug delivery process. Resistance of PA is partly due to a complex outer membrane (OM), which has low permeability. Substrate specific beta-barrel proteins mediate drug transport across the OM. It is this substrate specificity that provides the innate defense mechanism. Molecular dynamics (MD) and steered molecular dynamics (SMD) have lead to an increased understanding of the arginine transport pathway through the PA OM protein OccD1. Specific binding events thought to be key to transport have been observed; such as OccD1 loop movements and flipping of key residues. Additionally we have performed free energy calculations of a range of substrates across the PA OM to determine energetic barriers that must be overcome for permeation. Our results provide a key step towards developing novel PA antibiotics. A portion of this work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. LLNL-ABST-644304.



1293-Pos Board B23

Understanding Colicin N Import into Gram Negative Bacterial Cells using Small Angle Neutron Scattering

Wanatchaporn Arunmanee[1], Alexandra Solovyova[1], Christopher L. Johnson[1], Richard K. Heenan[2], Jeremy H. Lakey[1].
[1]Institute for Cell and Molecular Biosciences, Newcastle University, Newcastle upon Tyne, United Kingdom, [2]ISIS, Rutherford Appleton Laboratory, Didcot, United Kingdom.

Escherichia coli (*E. coli*) are gram-negative bacteria commonly found in human guts but which can cause food poisoning or severe systemic infections such as meningitis. Unlike other bacteria, gram-negative cells have an additional asymmetric outer membrane (OM) which consists of tightly-packed lipopolysaccharides in the outer leaflet of membrane and phospholipids on the inside. The OM is a selective permeability barrier and protects *E. coli* from antibiotics and bile acids. The occurrence of antibiotic-resistant bacteria is becoming increasingly serious and owing to the tightly-packed OM gram-negative bacteria can show even higher antibiotic resistance. In the search for novel effective antibiotics, Colicin N (ColN) is a promising model. ColN is a bacterial toxin produced and secreted by *E. coli* in time of stress.It translocates across the OM of target cells and kills via a voltage dependent channel in the inner membrane. ColN exploits outer membrane protein F (OmpF) as both a receptor and translocator. The pore-forming domain binds to the outside of OmpF (1, 2). Here we study the OmpF/ColN translocation complex in the presence of non-ionic detergents, where binding is driven by the translocation domain. The structure of complexes was determined by small angle neutron scattering using the SANS2D beam line and utilizing a contrast variation strategy with selective deuteration of proteins and Octyl Glucoside (OG). Mixing of three OG forms achieved exact contrast matches with the solvent. This enabled a low resolution structure of the translocon to be derived from *ab initio* modeling and revealed that the translocation and receptor-binding domains of ColN bind to the middle of the OmpF trimer.

1. Baboolal, T. G. *et al.Structure***16**, 371, (2008).
2. Clifton, L. A. *et al.J. Biol. Chem.***287**, 337,(2012).

1294-Pos Board B24

Affinities of Selective-Serotonin Reuptake Inhibitor (SSRI) for Human Transporters: Molecular Modeling and Quantum Chemical Studies

Krishna Deepak, Alok Jain, Ramasubbu Sankararamakrishnan.
Department of Biological Sciences and Bioengineering, IIT Kanpur, Kanpur, India.

SSRIs are the most commonly prescribed medication to treat mood, anxiety and personality disorders. SSRIs relieve symptoms of depression by selectively inhibiting the reuptake of the neurotransmitter serotonin from the synaptic cleft by the human serotonin transporter (hSERT). However, SSRIs also have weak affinities for the homologs of hSERT, the dopamine and norepinephrine transporters (hDAT and hNET). These promiscuous interactions of SSRIs with other neurotransmitter transporters can lead to severe side effects. A thorough understanding of the structural basis of SSRI-transporter interaction and selectivity is thus imperative for developing more potent and specific depression medication.

In this study, we constructed homology models of hSERT, hDAT and hNET bound to the SSRIs, namely, sertraline, R-flouxetine and S-fluoxetine. Five models were selected for each complex and protein residues within 3.5 Å of the bound SSRI were designated as the drug-binding residues. A total of 45 high-level quantum chemical calculations were performed using dispersion-corrected density functional theory (DFT-D3) and Ahlrich's triple-zeta basis set to estimate the interaction energies between drug-binding residues and SSRIs. Analysis of interaction energies clearly showed that hSERT exhibits the most favorable interaction energy for all three SSRIs. Analysis of non-covalent interactions between the protein and the inhibitors revealed the presence of additional stabilizing interactions in hSERT-SSRI complexes. Residues at structurally-equivalent positions in hNET and hDAT clearly lack those favorable interactions.

These calculations, for the first time, shed light on specific interactions responsible for SSRI selectivity in human neurotransmitter transporters. This knowledge can help in rational design of highly selective and more potent antidepressants.

1295-Pos Board B25

Structural Differences between the Closed and Open States of Channelrhodopsin-2 as Observed by Epr Spectroscopy

Nils Krause, Christopher Engelhard, Joachim Heberle, Ramona Schlesinger, Robert Bittl.
Experimental Physics: Genetic Biophysics, Freie Universität Berlin, Berlin, Germany.

Most ion channels are triggered by binding ligands or are voltage gated, whereas channelrhodopsin-2 (ChR2), a phototaxis receptor of algae, is a cation channel activated by light. This unique property is employed to optically trigger action potentials and other cellular activities. Besides its broad application in neurological research, light excitability makes ChR2 accessible to a broad range of spectroscopic techniques even on ultrafast timescales with the aim to address the functional mechanism of this unique membrane protein. The recently published structure on a ChR1/ChR2 hybrid resolved at a resolution of 2.3 Å provides a firm basis for probing structural changes at different positions during channel activity.

Two variants which contain either 1 or 2 wild-type cysteines were derivatised with nitroxide spin label and subjected to pulsed electron double resonance (pELDOR) spectroscopy. Both variants contained the C128T mutation to trap the long-lived P_3^{520} state by illumination as demonstrated by FTIR difference spectroscopy. Comparison of spin-spin distances in the dark state and after illumination reflect conformational changes in the conductive P_3^{520} state involving helices B and F. Spin distance measurements reveal that ChR2 forms a dimer in the absence of intermolecular N-terminal cysteine.

1296-Pos Board B26

Assessing the Transport Mechanism of Neurotransmitter Sodium Symporter Proteins with Molecular Dynamics

Emily Benner, Marco J. Acevedo, Jeffry D. Madura.
Duquesne University, Pittsburgh, PA, USA.

Neurotransmitter transporters, such as monoamine transporters (MATs), are membrane-bound proteins on the presynaptic terminal responsible for the reuptake of neurotransmitters from the synaptic cleft. Function of the MATs is associated with mood, emotion, and movement, among others. Dopamine transporter (DAT), a member of the MAT family, is responsible for the